

BOTANICAL GAZETTE

FEBRUARY 1900

STUDIES IN THE DEVELOPMENT OF THE POLLEN GRAIN IN SYMPLOCARPUS FÆTIDUS AND PELTANDRA UNDULATA.

B. M. DUGGAR.

(WITH PLATES I AND II)

DETAILS of the nuclear and cell division in the development of the pollen grain in these plants were first studied in *Symplocarpus fœtidus*, an abundance of favorable material having been secured and satisfactorily preserved during the winter of 1897-8. When it afterwards seemed well to study another plant in the same order, Professor Atkinson kindly put at my disposal material of *Peltandra undulata* which he had collected two years earlier. The latter material was also well fixed and preserved; but since it gave fewer developmental phases, *Peltandra* has been used largely by way of comparison with *Symplocarpus*. In general, these two plants agree in important phases of the cell activities, but there is considerable variation in some details.

The origin of the primitive archesporium was not studied in either plant, but in *Peltandra* there were found stages showing the divisions in the archesporial cells. These divisions are of the normal vegetative type; the number of chromosomes present, moreover, is that characteristic of the sporophyte.

THE RESTING ARCHESPORIAL CELL AND ITS NUCLEUS.

In *Symplocarpus* the cells of the definitive archesporium pass the late fall and early winter months in the resting condition.

Except in the size of the cells, no change was noticeable from early November to about January 1.

In the resting stage the cytoplasm is closely netted in the vicinity of the nuclear membrane, but there is no indication of a felt-like mass of kinoplasm. The nuclear membrane is more clearly defined than during the prophase of division. The nucleolus is more or less spherical, usually with at least one slight projection in view, the latter seeming to unite the nucleolus with the general reticulum. With gentian violet in the Flemming combination, the nucleolus stains a homogeneous purple; but with hæmatoxylin minute vacuolate appearances are often noted. Two or three smaller bodies resembling nucleoli may be present, and they are usually attached to distant parts of the reticulum.

At this time the reticulum is very slightly chromatic. It is loosely netted, with an indication of smaller knots at the anastomoses, and numerous small chromatin granules are scattered on the thread. In the reticulum stage, in fact, the nucleolus is the only nuclear structure which takes the chromatin stain freely, and very little indication of the safranin is retained after the use of the gentian violet.

SYNAPSIS.

Changes in the resting nucleus leading to the first evidence of a spirem and to the condition of synapsis have not been found abundantly. The threads of the reticulum become somewhat thicker, and the scattered granules upon it stain more deeply. Invariably these changes seem to precede that contracted condition of the linin network, or early spirem, well known as synapsis. Synapsis has been found abundantly at a definite period in the development of the pollen grains in these plants. In *Symplocarpus* there was no indication of this condition in material collected before the latter part of December; but for more than a month afterward it was often found, along with other stages, either later or earlier.

In both of these plants during the condition of synapsis the nucleolus becomes slightly wasted in appearance. The linin

framework contracts, at first irregularly. Finally it becomes closely pressed together, or "balled up," so that individual linin elements cannot well be distinguished, except where there are a few loose ends apparently serving for attachment to the nuclear membrane (*fig. 1*). The nucleolus is usually embraced in this contracted mass on one side, so that it protrudes prominently. Even in this condition, one or more slight projections on the nucleolus may sometimes be seen on the side near the contracted mass. The interwoven linin substance takes a diffuse stain. In the general mass there are also globules of a deeper staining substance. The nucleus does not become spread out against the wall, as described in some cases;¹ but it is usually situated on the side of the contracted mass distant from the wall. The contracted mass is either in contact with the nuclear membrane, or connected with the latter by linin threads.

During synapsis the cytoplasm becomes more loosely netted; and in *Symplocarpus* there is often an assemblage of kinoplasmic elements in that part of the cell which contains the greatest amount of protoplasm. These fibers are hardly so arranged as to be termed a cytoaster; and their general position may be either radial or oblique to the wall. A considerable number of large, deeply-staining granules are distributed in the cytoplasm. Another change which seems to be dependent upon synapsis is noted in the smaller nucleolar-like bodies of the nucleus. As separate bodies, any of these which may have existed in the resting condition disappear during synapsis, or they become closely adherent to the nucleolus and indistinguishable from the budded appearance of the nucleolus in the spirem, as mentioned later.

Synapsis has been found at a definite stage in the sporogenesis of many plants.² The marked differences noted in the stages

¹ SARGANT: The formation of the sexual nuclei in *Lilium Martagon*. I. Oogenesis. *Ann. Bot.* **1**: 457. 1896.

² Cf. a) FARMER: On spore formation and nuclear division in the Hepaticæ. *Ann. Bot.* **9**: 473, 481, 490. 1895.

b) STRASBURGER: Theilungsvorgang des Zellkerne, *figs. 3, 66*.

c) CALKINS: Chromatin-reduction and tetrad-formation in pteridophytes. *Bull. Torr. Bot. Club* **24**: *fig. 3*. 1897.

d) SARGANT: *l. c.*, also, II, Spermatogenesis. *Ann. Bot.* **11**: 187. 1897.

before and after this condition indicate that in some obscure way it must have an effect in the distribution of chromatin to the spirem ribbon. The changes evident on the return of the chromatin substance from synapsis are gradual but well marked. All phases of the return from this condition and leading to the spirem stage may be traced in a single anther.

It has been suggested by some that during the early prophase of division the cytoplasm is more difficult to fix, and from this difficulty may result the contraction termed synapsis. There is, however, little evidence for assuming that the cytoplasm is then so difficult to fix, nor is this condition a constant attendant of improper fixing during early prophase stages. The effect of diffusion currents, moreover, could not well produce such effects as found in *Symplocarpus*, since diffusion currents sufficiently strong to wrap the thread into a chromatic ball would hardly leave the nucleolus attached in the characteristic manner mentioned.

THE SPIREM STAGE AND SEGMENTATION.

On freeing itself from the contracted condition by a gradual loosening of the coils, the spirem skein in *Symplocarpus* takes the gentian readily, and it is easily traced as one or few coils with no anastomoses. The wide looping of the coil is more evident at this period than at any other. The thread is then made up of numerous adjacent disks of chromatin substance almost homogeneous with the linin connections. The chromatin disks become more distinct, and the ribbon spaces more noticeable; hence a somewhat nodulate appearance is presented, as in *fig. 2*. No evidence of a longitudinal splitting of the ribbon can be found at this time.

During the initiation of the spirem stage the nucleolus has a distinctly budded form, sometimes consisting of a single large body and of one or two smaller ones closely united, or two of nearly equal size. It is often very clear that the spirem thread is connected with these nuclear parts.

Following that stage in *Symplocarpus* where the spirem thread is uniform, there occurs a more abrupt bending and twisting of

the ribbon. Coincident with this, there is a thickening and a contraction in these looped regions. In some cases this thickening seems to be uniform; but generally there are very definite chromatin masses. Gradually these chromatin masses increase in size, and most of the chromatin disappears from the connecting regions.

The above changes lead to a condition very striking in appearance. At many places where thickening has occurred, there are several, usually about four, distinct chromatin aggregations (*fig. 3*). These are usually arranged in such a position as to suggest a division in two directions; hence a tetrad in the sense of the Freiburg school of zoologists. In many other instances there are distinct links open at one end, although the chromatin is in masses in these links. X and Y forms are much less common (*fig. 4*). A study of the changes leading to these conditions, however, seems to exclude the probability of tetrad-formation. Moreover, when viewed in the plane of the link there is indication of a longitudinal division in the linin connections between the chromatin masses, but never very marked. It is probable that the ribbon bends upon itself abruptly in the regions of the thickened chromatin masses to make the link forms, and that diverging forms are to be ascribed to a partial separation and reunion at the bend. If we accept this interpretation each chromatin group represents two chromosomes attached end to end and bent upon each other, with only a local indication of longitudinal fission, giving ultimately an effect such as others have described for *Lilium*; also for *Helleborus*, *Podophyllum*, *Iris*, and other plants. As these loops or chromatin groups increase in size, the indication of chromatin granules in the connecting linin diminishes. During early segmentation the prominence of these linin connections seems paralleled in certain *Hepaticae* studied by Farmer.³

During the aggregation of the chromatin into definite groups, important changes occur in the nucleolus. From the time when the large nucleolus is in close connection with one or more

³ *Loc. cit.*, p. 8, *figs. 5, 6, 41*.

smaller ones, the group gradually becomes fused, or the smaller bud-like processes disappear. The nucleolus is then drawn out in one or more directions so as to appear elliptical or triangular, or even rectilinear (*figs. 3, 4, 5*). It is, however, generally elliptical. Whatever may be the shape during this formative period of the chromosomes, the nucleolus is connected with certain linin threads, and it is evidently drawn out toward them. Often when the nucleolus appears circular in outline, by proper focusing certain attachments above or below may be seen. The fusiform and closely budded condition of the nucleolus was noted in living material, and I anticipated finding the *Sickelstadium* of Zimmermann.⁴

The chromosomes increase rapidly in size with the gradual disappearance of the chromatin from the linin connections. The incomplete union of the two segments in the process of bending back is often evident until the disappearance of the nuclear membrane, at which time the chromosomes tend to collect at the periphery of the nucleus. I have observed no protrusion of bundles of kinoplasm into the nuclear hollow before the separation of the fibers forming the nuclear membrane.

The spirem stage and the formation of the chromosomes are somewhat different in *Peltandra* from that which obtains in *Symplocarpus*, but I have fewer stages from which to draw conclusions. At no time can the spirem ribbon be distinguished as a loose coil. It twists and bends upon itself so freely as to suggest anastomoses (*fig. 15*). There are not such definite granules upon the ribbon, and the staining is much more homogeneous. Nothing which could be interpreted as a general longitudinal division of the ribbon was observed. On the other hand, it gradually becomes thicker where there seem to be anastomoses, and with further growth the whole extent of the skein is much shortened, and the chromatin thickenings are connected by slender threads, as in *fig. 16*. The nucleolus is often fusiform; but it never presents such exaggerated forms as in *Symplocarpus*. Each

⁴ZIMMERMANN: Über das Verhalten der Nukleolen während der Karyokinese. Beiträge z. Morph. u. Phys. d. Pflanzen.

nuclear segment bends upon itself, as in *fig. 17*, and with further growth the normal size is attained. The cytoplasm in *Peltandra* is very loosely netted, and in the vicinity of the nuclear membrane a marked radial arrangement of kinoplasmic fibers is evident during the late stages of chromosome formation, as has been abundantly described and figured (*figs. 17, 18*).

THE FIRST DIVISION.

On the disappearance of the nuclear membrane in *Symplocarpus* the kinoplasmic fibers entering the nuclear hollow are quickly oriented as a multipolar spindle with rather indefinite apices. The chromosomes are irregularly disposed on these fibers. The next most abundant stage is a multipolar spindle of very few poles, as in *fig. 6*. Finally a bipolar spindle is formed, the apices of which do not end in a very definite point (*fig. 8*).

When arranged upon the nuclear plate, it is difficult to interpret the exact form of the chromosomes in *Symplocarpus*, as they are so short and thick that fission lines and lines of union are almost indistinguishable. Looking down upon the chromosomes in an axial view, they seem to be somewhat quadrangular. A side view of a single segment shows the line along which separation will take place; and a polar view, *fig. 7a*, seems to indicate that the knee representing the original bend must be directed toward the center. It would be difficult at this stage to determine which plane represents the longitudinal fission and which the line of union of the doubled segment. The fission in a median plane is more distinct, and the external tips separate farther in this plane, as at *a*, *fig. 7*. Usually this is so evident, even before there is any separation of the daughter segments, as to make credible the view that they are so disposed as to separate qualitatively at the first division.

In *Peltandra* there is a less definite multipolar spindle than in *Symplocarpus*, although in the former the general form of a multipolar spindle is evident (*fig. 19*). With the completion of the bipolar form, the chromosomes are both centrally and peripherally disposed. The complete spindle is drawn together loosely

at the poles. There is a tendency for the polar cytoplasm to arrange itself radially.

The short chromosomes of *Peltandra*, which are sometimes of a short Y form are closely arranged on the nuclear plate, evidently with the knee inward. In the vicinity of the knee are attached the spindle fibers. If there is then any noticeable separation, indicating double V or Y forms, this separation is in the equatorial plane, thus giving the normal nuclear plate arrangement with longitudinal fission. The form of the chromosomes during the separation into the daughter segments (*fig. 20*) supports this view. The form of the short daughter segments soon after separation is somewhat oblong heart-shaped, since the angles of the V and Y forms seem to shorten and to fuse.

If, however, my interpretation of the condition of things in *Symplocarpus* is correct, the separation at the nuclear plate stage of the first division brings about a transverse division. Then, as suggested to me by Professor Atkinson, the closely following second division would provide for the separation of the segments marked out by the first division. Korschelt⁵ states that in *Ophryotrocha puerilis* reduction is effected during the first division.

There are about twenty-two chromosomes, and when they are arranged on the nuclear plate, a polar view may give the idea that they are variously fused. This appearance, however, is only due to the fact that the staining does not make evident the lines of contact, and a slightly oblique view aids the deception.

Radiations as of extra spindle fibers, which in some way are set free during the splitting of the chromosomes, veil the central spindle, and reach out almost to the periphery of the cell in the middle diameter of the spindle.

Cytoplasmic granules staining deeply are quite numerous in peripheral regions, and in *Peltandra* they are exceptionally large. In all stages of the developing and complete spindle, special attention has been given to the polar region, but in no case has there been any indication of bodies that might be taken for

⁵ Zeitschrift für wiss. Zoölogie 60 : 543-688.

centrosomes, although the cytoplasmic granules may appear in this region as well as in all other portions.

Although irregular in outline, in the daughter nuclei the chromosomes remain more or less distinct in *Symplocarpus*, and connected to one another by linin threads (*fig. 9*); while in *Peltandra* the chromosomes are more closely united into a dispirem ribbon, and not readily differentiated (*fig. 21*). In neither case is there any reappearance of the nucleolus before the second division is entered upon, this being in marked contrast with what obtains in certain dicotyledons under study at the same time—especially *Bignonia*.

In the telophase of division in *Peltandra*, the kinoplasmic fibers largely disappear from the middle portion of the cell after the formation of the cell plate has begun, and these fibers are then found to be abundantly concentrated at the periphery. They pass around in the plane of the long axis of the nucleus, entirely surrounding the latter. They form a complete web around the periphery of the daughter cells, especially abundant in the equatorial region (*fig. 21*). When the development of the cell plate is complete, and the two daughter cells are distinct, these radiations are separated at the extreme edge; and from that point they radiate as before (*fig. 22*). They are evidently concerned in the formation of an independent wall, even in the two-cell stage.

THE SECOND DIVISION.

After the first division in *Symplocarpus*, some peculiar condition renders staining a very difficult process. The safranin-gentian-orange gives a diffuse pink in the general cytoplasm, and the chromosomes appear indefinite in outline. Iron haematoxylin often works to better effect if the sections are deeply stained and much decolorized. In this stage it is necessary to make the sections very thin. The difficulty in staining persists until the microspores become free, and then again the material is favorable. The second division has not been studied in *Peltandra*.

With the disappearance of the nuclear membrane, there is formed a clearly defined multipolar spindle, whose general axes from the first are perpendicular to the plane of the first division. This readily becomes bipolar in the usual manner. The form of the chromosomes as arranged on the nuclear plate is more or less quadrangular in front view, and oblong as observed from the poles. Viewed axially, there is an evident line of splitting, and on fission there result short, rod-shaped, daughter segments. The character of this division, of course, depends largely upon the character of the first division, and the doubt involved in this first division cannot now be cleared up.

After the formation of the four nuclei denoting the future microspores, there is a slight reappearance of spindle connections between the daughter cells, or, rather, secondary spindles are differentiated with very delicate spindle fibers.

THE MICROSPORE.

Upon the dissolution of the mother-cell membrane, the tapetal cells become free, and wander in between the maturing microspores. Coming in contact with these forming microspores, the tapetal cells lose their identity as distinct cells, forming a general protoplasmic stratum, in which the nuclei persist for some time. Imbedded in this nourishing layer, the microspores rapidly increase in size, at the same time assuming a thicker wall.

When first freed from the mother-cell membrane, the nuclei of these microspores do not show a single definite nucleolus. Instead, there are usually several irregular masses, which constitute the chromatin content. With the maturity of the spores, a single large nucleolus, more or less spherical in form, is evident, although there may be one or more much smaller bodies staining similarly.

DIVISION OF THE MICROSPORE NUCLEUS.

In neither plant has the spirem stage of this division been studied in detail. In *Symplocarpus* the earliest preparations of

the spirem show numerous distinct chromatin granules on a comparatively short ribbon. The ribbon soon becomes much thickened, with many sudden bends and irregular twists, larger chromatin masses being evident at definite places. By its bendings the whole ribbon seems to be marked off into definite segments, as in *fig. 10*. At this stage often the connection of the ribbon with the nucleolus may be seen. Immediately preceding actual segmentation, as well as afterward, each forming segment has its chromatin massed toward the ends. A longitudinal splitting of the segment is then evident, thus somewhat imitating a characteristic of heterotypic division. On separation, or complete segmentation, the form of the chromosomes is that of a double half link.

With approaching division, the nuclear cavity increases somewhat in size, and the chromosomes are in the vicinity of the nuclear membrane. The nucleus is in a position near that wall, along which, apparently, the microspore was adherent in the mother cell. With the gradual opening of the network forming the nuclear membrane, kinoplasmic threads enter the nuclear hollow largely from the region of the greatest cytoplasm; and no previous radial arrangement of these threads is noticeable.

In the forming spindle of the microspore nucleus, an interesting variation from the normal type of multipolar spindle prevails. The fibers readily take up a position more or less perpendicular to the wall near which the nucleus is located. These fibers arrange themselves in contact with this wall at many points, or in a subpolar manner. At the opposite or free end there are at first, also, false poles. There is then a multipolar spindle in an axial plane. Finally, at the free end, the clusters are usually drawn together into a single pole, or what is approximately a single pole. The complete spindle is thus truncated at one end, and more or less conical at the other (*fig. 11*).

When arranged in the nuclear plate, the ends of the chromosomes point outward, and on account of the loop form there appear to be more chromosomes in the periphery than are really present. As in the case of the pollen mother-cell divisions,

the chromosomes are loosely arranged throughout; and the metaphase proceeds with separation of the daughter segments at the split previously indicated. In the anaphase, when drawn to the fixed pole, the chromosomes are arranged as if in close contact with the membrane adjacent.

From the position of the microspore nucleus just previous to this division, it is to be noted that the spindle is further peculiar on account of its orientation in a plane perpendicular to the greatest mass of protoplasm. There is some evidence that the nucleus of the microspore constantly remains in the vicinity of that wall of the microspore which was in contact with the other spores in the group of four, yet certainly not in contact with this wall. Since this wall is usually in a plane perpendicular to the greatest mass of protoplasm, this may partially account for the constant orientation of the spindle. The free pole of the complete spindle occupies approximately the center of the spore, hence the axis of the spindle is about equal to half the short diameter of the spore. In this division the sudden disappearance of the large nucleolus is a matter of some interest, in connection with other observations previously given upon this organ. In some cases large fragments of nucleolar-like substance seem to be entangled in the fibers of the developing spindle, and in several cases a large mass of this material has been found at the free pole, and apparently held between the fibers.⁶ Moreover, in accordance with the observations of many others, I noticed only a few deeply staining granules in the cytoplasm previous to the disappearance of the nucleolus; but in the anaphase and telophase stages of division they are especially abundant in the vicinity of the free pole. They do not, however, disappear with the formation of the daughter nuclei, persisting for a time, but disappearing almost entirely before the pollen grain is mature.

The general effect of the division of the microspore nucleus is well known, but some details may be mentioned, particularly

⁶DEBSKI: Beobachtungen über Kerntheilung bei *Chara fragilis*. Jahrb. f. wiss. Botanik 30: 227. 1897.

concerning the cell division. Invariably a cell plate separates the daughter nuclei, and the concave side of the forming membrane is toward the small, generative nucleus (*fig. 12*). Generally the generative cell is cut off by a distinct wall, and in the pollen grain two principal types of generative cells are seen. In the first place, the strongly convex membrane necessitates the formation of an oval cell, and the large vegetative cell grows closely around it. The smaller cell is, as it were, drawn into the protoplasm of the surrounding cell, and thus the former becomes more nearly spherical in outline. The generative cell at first contains considerable cytoplasm, but this is gradually lost, although the shrunken wall remains, as in *fig. 13*. Whether taken into the protoplasm or not, the space between the walls of the vegetative and generative cells is evident, and remains surrounding the generative cell as a distinct court. On the other hand, the generative cell may remain closely adherent to the wall for some time, and in this case it is sometimes oval, but usually distinctly fusiform or lens-shaped, sooner or later. The fusiform cell usually remains attached until germination of the pollen grain. As before, the cytoplasm largely disappears, and there remain only small courts on each side of the oblong nucleus. In some cases the nucleus itself becomes fusiform, and it is difficult to distinguish the enveloping walls.

In the division of the microspore nucleus in *Peltandra*, the spirem ribbon is longer and more complicated than in *Symplocarpus*; and on segmentation the chromosomes are longer. As the daughter segments pass to the pole, they are distinctly U or V-shaped, as in *fig. 24*. The spindle is formed much as in *Symplocarpus*, being somewhat multipolar at the pole which is in contact with the wall layer; and at the free pole the apices do not closely converge, as shown in *fig. 23*. In *Peltandra*, moreover, it is not such an open question as to the influences determining the position of the nucleus in the mature microspore. A large vacuole occupies much of the contents of the microspore, and this seems to be an efficient means for the orientation of the nucleus in such a position that one pole of the spindle

may be in close contact with the wall of the spore; the resulting division gives again the characteristic difference in the size of the cells. In this case, however, the plane of the spindle is usually in the plane of the long diameter of the spore.

With the orientation of the microspore spindle so fixed as it is in these plants, it is easy to explain the dissimilarity in the size of the resulting cell-bodies. In this division, moreover, there is evidently equal division of the chromatin, as there is in all normal karyokinesis. The smaller nucleus resulting can have nothing to do with the chromatin content, and must depend upon its relation to the smaller cell body. From the zoological point of view, unequal segmentation as a factor in differentiation is fundamental, and various speculations have been entered upon as to the cause of unequal division of the cell-body. Wilson⁷ prefers Conklin's⁸ view, that the cause lies in some relation between the karyokinetic figure and the cell body in which it lies. The inequality in the sizes of the nuclei in the pollen grain, moreover, might be explained largely from the unequal cell divisions. In those cases, however, of division in the microspore where it is reported that no cell wall is formed, we must assume that at least a transient plate or membrane must be formed in order to regulate the sphere of activity about each nucleus, and hence, probably, the size of each nucleus.

In *Symplocarpus* there was no division of the generative nucleus in the pollen grain. Germinated pollen was obtained in a few hours by sowings on pith in 3 per cent. sugar solution. On a moist day I also found germinated pollen in the bract of the flower, but this is unsatisfactory to work with on account of the difficulty of taking it through the embedding process. I did not secure spindle stages in the division of the generative cell, but enough was secured to show that division occurs soon after the nucleus passes into the pollen tube. The vegetative nucleus usually passes into the pollen tube first and does not deteriorate until after the division of the generative cell. The generative

⁷ The cell in development and inheritance 275.

⁸ The fertilization of the ovum. Biol. Lect. Marine Biol. Lab. Boston, 1894.

nucleus passed into the pollen tube while still inclosed within its cell membrane; and several preparations showing stages following division indicate that a membrane surrounds the two nuclei for some time (*fig. 14*). From the same figure it will be seen that the chromosomes of the daughter nuclei have not formed a dispirem, but remain for some time in the segmented form. What the form of these nuclei may be at a later stage, I have not been able to determine.

NOTES ON MATERIALS AND ON METHODS.

Collections.—In *Symplocarpus* I failed to secure any material earlier than October 1, and at this time the definitive microsporic archesporium was formed, and the nuclei of the pollen mother cells were well in the resting condition. The resting condition was found as late as January 1; but much of the material collected during late December and early January was in synapsis. Division of the pollen mother-cells occurred abundantly during the first warm days of February. Although a true spike, the order of maturity of the anthers in *Symplocarpus* is acropetal, in spite of the fact that the receptivity of the stigmas is often synchronous. It is essential in collecting material to make an examination of a median anther to ascertain the general stage of development, although in exceptional cases there may be great variation in a single spike. It is not uncommon to find all stages from an early spirem to a late stage of division in the pollen mother-cells. However, the development seems to go somewhat in waves. Usually all are in synapsis at the same time; then there is a rest after the second division of the pollen mother-cells, and there is no actual division of the microspore nucleus in any anther until at least the spirem stage of this division has been reached by all.

For the best penetration of the fixative, it is necessary to open the anthers, and this is readily effected wholesale by shaving off a little of the outer portion of the spike. The stamens may be again cut off next to the surface of the axis, and readily separated from the parts of the perianth. After fixing twenty-four

hours, the material was washed for a few hours, and then carried through several grades of alcohol to 70 per cent. It was necessary to decolorize with peroxide of hydrogen, and this can be done advantageously by mixing it with alcohol to a strength of 70 per cent. alcohol.

After dehydration with absolute alcohol, cedar oil has been used as an infiltrating agent. A small quantity is poured into the absolute alcohol, and into this the material soon sinks. When clear, a change to pure cedar oil is made for a number of hours, then into a mixture of paraffin and cedar oil, and finally into pure paraffin for a few hours. A change of the pure paraffin is well, in order to rid the tissues of all cedar oil; for a trace of the latter may cause electrification and crushing in section cutting.

Staining.—A modification of Flemming's triple stain of safranin, gentian violet, and orange was relied upon primarily, but quite generally supplemented with iron haematoxylin and orange. In using the triple stain, various safranins were used; but with *Symplocarpus* a final effect of the safranin was of no value, and it was quite generally discarded for a double stain of gentian violet and orange. Ehrlich's gentian violet was used, and for some stages short staining with the strong solution would suffice; but in cases where there was difficulty in staining the spindle fibers, or cytoplasmic radiations, it was found much better to stain from eighteen to twenty-four hours in a tumbler of water containing about one cubic centimeter of the gentian. The orange was used from a fraction of a minute with spindle stages to several minutes with mature pollen grains. After the orange, I have found it advisable to wash out most of the surplus gentian in absolute alcohol, or rinsing at first with 95 per cent. alcohol, if desired. Differentiation with clove oil was effected by simply dashing the slide with the oil, since a much longer action often causes a loss of detail. The sections are then fixed and cleaned in bergamot oil before mounting. In some cases it is well to dash with bergamot oil before differentiating the orange with clove oil, especially in stages where the gentian is

very readily lost; for in such stages clove oil must be used very cautiously. In plants whose cytoplasmic structure is very loosely netted, these precautions are unnecessary. In general, I have secured better differentiation of the chromosomes, or rather more distinct outlines, by using strong gentian. On the other hand, spindle effects are usually superior from the use of dilute stains. Considerable experimentation is necessary in order to determine what is the best method of procedure for each plant studied; but for this purpose time may well be afforded.

Iron hæmatoxylin gave some brilliant results where the Flemming combination was least effective, as in the second division of the pollen mother-cells of *Symplocarpus*, and especially in stages of the maturing pollen grain and of the dividing microspore nucleus. It was found well to keep the sections in the iron alum for more than an hour, and then to stain in hæmatoxylin from twenty-four to thirty-six hours, from which a better differentiation results. Otherwise, it is difficult to get such a distinct effect in the chromosomes when the cytoplasm is properly decolorized.

BOTANICAL DEPARTMENT,
CORNELL UNIVERSITY.

EXPLANATION OF PLATES I AND II.

PLATE I. Symplocarpus fœtidus.

All figures drawn with the aid of the camera lucida, at 12 ins. projection, tube length 155^{mm}; nos. 1-12, 14-19, and 21-24 with comp. ocular 8 and $\frac{1}{18}$ hom. imm.; no. 13, ocular 3 and objective as before; no. 20, comp. ocular 18 and objective as before.

FIG. 1. Nucleus in synapsis; peculiar orientation of kinoplasm in the cytoplasm.

FIG. 2. Spirem stage in which the chromatin disks are prominent on the linin framework.

FIG. 3. Early development of the chromosomes; chromatin appearing in tetrad-like groups; fusiform nucleolus.

FIG. 4. A segmentation stage in which loops and X and Y forms are abundant.

FIG. 5. Forms of nucleoli not infrequently observed.

FIG. 6. Multipolar spindle with only a few prominent poles.

FIG. 7. Complete spindle of the first division, showing the form of the chromosomes on the nuclear plate; 7a, a polar view of chromosomes as arranged on nuclear plate.

FIG. 8. Pollen mother-cell during an early anaphase of division.

FIG. 9. Daughter nuclei with chromosomes distinct, but irregular in form; early stage of cell-plate formation.

FIG. 10. Microspore nucleus in an early stage of segmentation.

FIG. 11. Peculiar spindle of the microspore division.

FIG. 12. Daughter nuclei resulting from the division of the microspore nucleus.

FIG. 13. Pollen grain with small generative cell surrounded by its court, but free in larger vegetative cell.

FIG. 14. Pollen tube showing sperm nuclei and deteriorating vegetative nucleus.

PLATE II. *Peltandra undulata*.

FIG. 15. Nucleus in spirem stage.

FIG. 16. An early stage in the segmentation of the chromosomes.

FIG. 17. Radial arrangement of kinoplasm in pollen mother-cell; chromosomes differentiated.

FIG. 18. Pollen mother-cell with kinoplasmic weft in the vicinity of the nuclear membrane at the time when the latter begins to disappear.

FIG. 19. A stage in the development of the rather indefinite multipolar spindle; chromosomes occupying a central position.

FIG. 20. Complete spindle of the first division, also showing the separation of the segments.

FIG. 21. Daughter nuclei resulting from the first division of the pollen mother-cell; cell plate forming at the periphery.

FIG. 22. Daughter cells showing the peculiar arrangement of the kinoplasm at the completion of the cell division.

FIG. 23. A division of the microspore nucleus, showing the characteristic spindle.

FIG. 24. An anaphase stage in the division of the microspore nucleus.